

Catellatospora citrea subsp. *methionotrophica* subsp. nov., a Methionine-Deficient Auxotroph of the *Actinomycetales*

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Two isolates belonging to the genus *Catellatospora* were recovered from soil samples collected at different geographical sites in Japan. These isolates showed many similarities to the type strain of *Catellatospora citrea* in chemotaxonomy and morphology, but they required an external source of methionine for growth. They are described as a new subspecies of *Catellatospora citrea*, *Catellatospora citrea* subsp. *methionotrophica*; the type strain is strain IFO 14553 (= 6257-B).

In 1986 we proposed *Catellatospora*, which is a genus belonging to the Actinoplanetes group in the *Actinomycetales* (1) and which forms chains of nonmotile spores from vegetative hyphae on the surface of agar media, lacks true aerial mycelium, and has type II cell wall amino acids (*meso*- and 3-hydroxy diaminopimelic acids and glycine) (3), a glycolyl type of cell wall sugar (7), a pattern D whole-cell sugar (xylose and arabinose) (2), and a type PII phospholipid pattern (phosphatidylethanolamine) (1, 4).

We have isolated two additional strains of this genus, strains 6498-D and 6257-B^T (T = type strain), from woodland soil samples collected at different geographical sites in Japan. These isolates satisfied the characteristics of the genus *Catellatospora*. They contained MK-9(H₄) as the major menaquinone (Table 1), formed straight chains of smooth-surfaced spores, and had yellowish vegetative hyphae without any soluble pigment production. Table 2 shows the culture characteristics, as determined by the method of Shirling and Gottlieb (5). These properties are similar to the characteristics of the previously described species *Catellatospora citrea* (1).

The physiology of the new isolates was compared with that of the type strain of *C. citrea*, strain 6183-E (= IFO 14495). These strains grew in a neutral pH range and under mesophilic conditions (20 to 34°C), were not able to grow on media supplemented with 3% NaCl, and were susceptible to novobiocin (50 µg/ml), vancomycin (50 µg/ml), gentamicin (50 µg/ml), demethyltetracycline (500 µg/ml), and streptomycin (100 µg/ml). Carbon compound utilization was compared on Bacto yeast nitrogen base basal medium (Difco Laboratories) neutralized with 20% (vol/vol) 1% K₂HPO₄. Utilization was positive for D- and L-arabinose, cellobiose, D-galactose, D-glucose, lactose, maltose, D-mannose, L-rhamnose, starch, sucrose, and D-xylose and negative for ethanol, *meso*-erythritol, D-fructose, gluconate, *myo*-inositol, D-mannitol, melezitose, methanol, raffinose, and D-sorbitol.

The two new isolates and the type strain of *C. citrea* utilized the same carbohydrates (see above). Strain 6498-D differed from the type strain of *C. citrea* by not utilizing melibiose, α-methyl-D-glucoside, D-ribose, or salicin. Strain 6257-B^T differed in only one carbon source; it did not utilize melibiose. The most significant difference between the two new isolates and the type strain of *C. citrea* was that isolates 6498-D and 6257-B^T required methionine for growth, which

TABLE 1. Menaquinone compositions of *C. citrea* subsp. *methionotrophica* strains^a

Strain	% Content of:						
	MK-7		MK-8		MK-9		
	H ₄	H ₆	H ₄	H ₆	H ₄	H ₆	H ₈
6257-B ^T			6	2	66	21	4
6498-D	4	1	2	1	60	22	9

^a Menaquinone composition was analyzed by the method of Tamaoka et al. (6).

could not be replaced by cysteine or homoserine. Because of this nutritional requirement, the isolates grew poorly or not at all on synthetic agar media, such as sucrose-nitrate, glucose-asparagine, glycerol-asparagine, or salts-starch medium.

On the basis of the data described above, we regard isolates 6498-D and 6257-B^T as a new subspecies of *C. citrea*, for which we propose the name *Catellatospora citrea* subsp. *methionotrophica*; strain 6257-B is the type strain of the new subspecies. A description is given below.

Catellatospora citrea subsp. *methionotrophica*. subsp. nov. *C. citrea* subsp. *methionotrophica* (methio.no.tróphi.ca. N.

TABLE 2. Cultural characteristics of *C. citrea* subsp. *methionotrophica* strains^a

Agar medium	Strain 6257-B ^T		Strain 6498-D	
	Growth	Color of reverse side of colony ^b	Growth	Color of reverse side of colony
Nutrient	Poor	Colorless to apricot (4 ga)	Moderate	Bright gold (2 pc)
Yeast extract-malt extract	Good	Light apricot (4 ea)	Moderate	Amber (3 lc)
Oatmeal	Poor	Light wheat (2 ea)	Poor	Mustard gold (2 ne)
Glucose-yeast extract	Poor	Colorless	Poor	Dusty orange (4 lc)
Hickey-Tresner	Good	Bright marigold (3 pa)	Moderate to good	Amber (3 pc)

^a Cultures were incubated at 28°C for 3 weeks. The isolates did not grow on synthetic media (sucrose-nitrate, glucose-asparagine, glycerol-asparagine, and salts-starch media). Aerial mycelium was not formed on any of the media.

^b The colors were determined as described in reference 1.

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Lat. adj. *methionotrophica*, methionine auxotroph). Most of the characteristics are similar to those of *C. citrea* (1), except for an absolute requirement for methionine and no utilization of melibiose. Isolated from a soil sample collected in Yamashashi, Japan. The guanine-plus-cytosine content of the deoxyribonucleic acid is 70.8 ± 0.5 mol%. The type strain of *C. citrea* subsp. *methionotrophica* is strain IFO 14553 (= 6257-B).

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